

Protein Hydrolysate from Miscellaneous Fish

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A method to prepare fish protein hydrolysate from miscellaneous fish obtained as by catch from shrimp trawlers is outlined. Effect of temperature and concentration of enzyme papain on the yield of hydrolysates has been determined. It is seen that within 30 min at 55°C and pH 6.5 fish proteins can be effectively solubilised, provided the nitrogen content of the enzyme (activity 10 units/mg enzyme) and substrate are maintained in the ratio 1:30. This hydrolysate possesses the best amino acid pattern compared to those obtained after hydrolysis for 60 to 180 min.

Miscellaneous fish constitute a major portion of the fish catch. At present they are not utilised fully and some attempts have been made to utilise them (Anon, 1973, '76, '77, '78). Protein hydrolysate from miscellaneous fish (trash fish) has several commercial applications. Dietary protein is absorbed in the form of oligo peptides and free amino acids. Protein hydrolysates which contain oligo peptides and free amino acids have better physiological advantages in correcting malnutrition compared to a mixture of free amino acids (Mathews, 1971; Silk, 1974).

Fish protein has been used as raw material for the production of hydrolysates by many workers. The factors affecting the degree of hydrolysis and the fractional analysis of certain papaic hydrolysates of fish flesh have been reported by Sen *et al.* (1962) and Sripathy *et al.* (1962). Sripathy *et al.* (1963) showed that fish hydrolysates are of good nutritional value. Technological aspects relating to the production of hydrolysate from a fresh water fish, *Barbus dubius* using papain have been worked out by Sripathy *et al.* (1964). Hale (1969) measured the relative activities of more than twenty commercially available proteolytic enzymes for the digestion of a washed and freeze-dried fish protein substrate and showed that pepsin, papain and pancreatin all combined good activity with moderate cost. Hale (1972) has also reported effects of various processing conditions and commercially available proteolytic enzymes on yield and composition

of water soluble fish protein hydrolysates. According to McBride *et al.* (1961) pepsin produced better solubilization of herring tissue than other proteolytic enzymes. Yanez *et al.* (1976) employed hake as raw material for the production of protein hydrolysates and used it for the supplementation of cereal protein. However, one of the disadvantages of protein hydrolysate is its bitterness, caused by the presence of bitter peptides. Recently, Lalasidis *et al.* (1978) and Lalasidis & Sjoberg (1978) used a powerful bacterial endopeptidase alcalase, followed by pancreatin to produce bitter-free protein hydrolysate from deboned cod filleting offal, fish protein concentrate and fresh herring. Both pepsin and papain were initially tried to produce hydrolysates by the authors. Subsequently pepsin was discontinued from the studies as it involved hydrolysis at a lower pH, which necessitated the neutralisation of the final product thereby increasing concentration of salt in the finished product. The physical properties of peanut flour hydrolysed by bromelin, pepsin and trypsin have been reported by Beuchat *et al.* (1975), but pepsin and trypsin are too expensive for commercial applications. Papain is reported to be more desirable in proteolysis than bromelin (Sekul & Ory, 1977). Sekul *et al.* (1978) also showed that proteolysis of peanut proteins with papain caused certain functional changes in the properties of hydrolysed proteins with certain advantages in food applications. The present study reports the effect of temperature, time and concentration of

Table 3. *Effect of temperature on hydrolysis of minced whole fish (enzyme nitrogen to substrate nitrogen ratio 1:30, pH 6.5, time of hydrolysis 30 min)*

Sample	Yield * of hydrolysate at				
	40°C	50°C	55°C	60°C	70°C
1	9.0	10.8	11.3	10.7	10.0
2	10.7	11.5	12.9	11.3	10.7
3	10.5	12.2	12.8	12.4	12.3
4	8.7	8.8	11.1	9.3	8.9

* g/100g whole fish

Table 4. *Amino acid composition of protein hydrolysates at different times of hydrolysis (enzyme nitrogen to substrate nitrogen ratio 1:30, pH 6.5, temperature 55°C)*

Amino acids*	Hydrolysate			
	30 min	60 min	120 min	180 min
Lysine	7.32	8.34	7.10	7.92
Methionine	2.67	2.54	2.71	3.20
Leucine	3.03	2.14	3.16	3.21
Isoleucine	2.36	3.14	3.43	3.11
Threonine	5.11	4.96	4.93	4.87
Tryptophan	0.99	0.89	0.79	0.84
Phenylalanine	4.43	4.39	4.41	4.06
Valine	2.26	1.96	2.43	2.37
Arginine	4.25	5.31	5.52	5.67
Glutamic acid	10.51	13.56	13.60	13.41
Aspartic acid	4.62	4.91	5.26	5.71
Histidine	2.51	3.91	5.73	4.97
Glycine	2.50	2.91	2.46	2.62
Serine	4.75	5.25	4.95	4.31
Proline	3.61	4.66	4.21	3.95
Cystine	0.47	0.63	0.62	0.69
Tyrosine	4.81	5.24	5.40	5.16

* g/100g protein

nitrogen were in the ratio 1:30. Using this ratio, the effect of reaction time on proteolysis was worked out (Table 2). Though maximum yield of hydrolysate was obtained when hydrolysis extended up to 180 min the differences in yield was not appreciable compared to that at 30 min. Further reduction in time of hydrolysis to 15 min entailed in certain cases appreciable lowering in the yield of hydrolysate, suggesting that a viable yield could be obtained after 30 min hydrolysis.

The study of the effect of temperature on proteolysis of fish proteins with enzyme

papain shows that (Table 3) an operational temperature of 55°C will be the most suitable for the maximum yield of hydrolysate.

The study of the overall amino acid pattern of the different hydrolysates produced at 30, 60, 120 and 180 min showed very little variation in the individual amino acid make up. However, it should be noted that tryptophan, phenylalanine and threonine are lost significantly when hydrolysis was extended to 180 min. Glycine, proline, leucine and valine showed very little change between 30 and 180 min. The rest of the amino acids showed a

Table 5. Yield of protein hydrolysate from miscellaneous fish (enzyme nitrogen to substrate nitrogen ratio 1:30, pH 6.5, temperature 55°C, time of hydrolysis 30 min).

Common name	Species name	Yield* of hydrolysate
Lizard fish	<i>Saurida tumbil</i>	13.3
Large spined flat head	<i>Platycephalus macracanthus</i>	11.0
Ribbon fish	<i>Trichiurus</i> sp.	9.9
Barracuda	<i>Sphyræna</i> sp.	11.9
Jew fish	<i>Johnius</i> sp.	9.9
Threadfin bream	<i>Nemipterus japonicus</i>	12.0
Cat fish	<i>Tachysurus</i> sp.	10.9
Anchovies	<i>Thrissocles</i> sp.	9.7
Sole	<i>Cynoglossus</i> sp.	8.6

* g/100g whole fish

marginal increase. Considering the loss of tryptophan, phenylalanine and threonine (essential amino acids) it is not advisable to carry out hydrolysis up to 180 min from the nutritional point, as they will become limiting amino acids when protein scores are evaluated to incorporate hydrolysates in food preparations. It is also reported that extended enzymatic digestion of fish proteins causes a reduction of their nutritional value and a partial destruction of essential amino acids (Gillies, 1975). Thus the best amino acid pattern is obtained in the product after 30 min hydrolysis.

Picked fish meat, obtained with the help of a mechanical flesh separator, which removes bones, scales and skin from meat, invariably gave lower yield compared to whole fish meat. The higher yield of hydrolysates from whole fish can be interpreted as due to the presence of calcium ions in the meat, obtained from bones, which is found to activate papain.

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References

Anon (1973) *Proc. ii Workshop, Indian Council of Agricultural Research Co-ordinated Research Project on Transportation of Fresh Fish and Utilization of*

Trash Fish. Mangalore, April 5-8 (unpublished)

Anon (1976) *Proc. IV Workshop, Indian Council of Agricultural Research Co-ordinated Research Project on Transportation of Fresh Fish and Utilization of Trash Fish.* Cochin, October 27-28 (unpublished)

Anon (1977) *Proc. V Workshop, Indian Council of Agricultural Research Co-ordinated Research Project on Transportation of Fresh Fish and Utilization of Trash Fish.* Madras, November 15-16 (unpublished)

Anon (1978) *Proc. VI Workshop, Indian Council of Agricultural Research Co-ordinated Research Project on Transportation of Fresh Fish and Utilization of Trash Fish.* Mangalore, November 3-4 (unpublished)

Beuchat, L.R., Cherry, J.P. & Quinn, M.R. (1975) *J. agric. Fd chem.* **23**, 616

Gillies, M. T. (1975) *Fd Technol. Rev.* **22**, 313

Hale, M.B. (1969) *Fd Technol.* **23**, 107

Hale, M.B. (1972) *NOAA Technical Report NMFS SSRD 657, Making Fish Protein Concentrates by Enzymatic Hydrolysis*

- Kavanagh, F. (1963) *In Analytical Microbiology*, 1st edn., p. 167, Academic press, New York
- Lalasisdis, G., Bostrom, S. & Sjoberg, L.B. (1978) *J. agric. Fd chem.* **26**, 751
- Lalasisdis, G. & Sjoberg, L.B. (1978) *J. agric. Fd chem.* **26**, 742
- Mathews, D.M. (1971) *J. Clin. Pathol.* **24**, Suppl. (Roy. Coll. Path) **5**, 29
- McBride, J.R., Idler, D.R. & Macleod, R.A. (1961) *J. Fish. Res. Bd Can.* **18**, 93
- Nanda, C.L., Ternmouth, J.H. & Kondas, A.C. (1977) *J. Sci. Fd Agric.* **28**, 20
- Sekul, A.A. & Ory, R.L. (1977) *J. Am. Oil chem. Soc.* **54**, 32
- Sekul, A.A., Vinnet, C.H. & Ory, R.L. (1978) *J. agric. Fd chem.* **26**, 855
- Sen, D.P., Sripathy, N.V., Lahiry, N.L., Sreenivasan, A. & Subramanyam, V. (1962) *Fd Technol.* **16**, 138
- Silk, D.B.D. (1974) *Gut.* **15**, 494
- Sripathy, N.V., Sen, D.P., Lahiry, N.L., Sreenivasan, A. & Subramanyam, V. (1962) *Fd Technol.* **16**, 141
- Sripathy, N.V., Kadkol, S.B., Sen, D.P., Swaminathan, M. & Lahiry, N.L. (1963) *J. Fd Sci.* **28**, 365
- Sripathy, N.V., Sen, D.P. & Lahiry, N.L. (1964) *Res. & Ind.* **9**, 258
- Yanez, E., Ballester, D. & Monckeberg, F. (1976) *J. Fd Sci.* **41**, 1289